Cryptogamie, Mycol. 1988, 9 (2) : 157-165

CONTROL GROWTH OF WOOD-ROTTING FUNGI BY NON-VOLATILE METABOLITES FROM *TRICHODERMA* SPP. AND *GLIOCLADIUM VIRENS*

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SUMMARY – *Trichoderma* spp. and *Gliocladium virens* isolated from grassland soil were tested for growth inhibition of 10 wood-rotting Basidiomycetes. *T. hamatum* was the most active inhibitor against the Basidiomycetes and the only one producing growth stimulation at least for one species. Growth inhibition of many Basidiomycetes decreases with time. This was clearly correlated with antagonistic metabolites diffusion in agar. There were few species that failed to show clear inhibition with some of the antagonistic moulds. *Fomes pomaceus*, one of the most sensitive species, was completely inhibited by *G. virens* at a relative high metabolite concentration : the inoculum transfered to fresh medium culture failed to develop.

RÉSUMÉ – L'activité inhibitrice des métabolites non-volatiles de *Trichoderma* spp. et de *Gliocladium virens*, isolés à partir d'un sol de prairie, a été testée sur 10 souches de Basidiomycètes lignivores. *T. hamatum* est l'inhibiteur le plus actif vis-à-vis de ces souches et est le seul à stimuler la croissance d'au moins une des espèces. L'inhibition diminue avec le temps et est corrélée avec la diffusion des métabolites dans le milieu de culture. Peu de souches sont insensibles à l'effet inhibiteur des moisissures, la plupart montrent une sensibilité très nette. *Fomes pomaceus*, une des espèces les plus sensibles, est complètement inhibée par *G. virens*, à des concentrations de métabolites relativement élevées.

KEY WORDS : wood-rotting fungi, *Trichoderma* spp., *Gliocladium virens*, growth control, non-volatile metabolites.

INTRODUCTION

Trichoderma spp. as effective control agents of several phytopathogens (CHET, 1987) and of some wood-rotting Basidiomycetes (RICARD, 1977; BRUCE & KING, 1983; BRUCE & al., 1984) have been confirmed. Less consideration has been given to the activity of *Gliocladium virens* Miller, Giddens & Foster perhaps due to the confusion prevalent at regards the identity of this fungus (WEBSTER & LOMAS, 1964; DENNIS & WEBSTER, 1971a), since we

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are dealing with a species less frequent than *Trichoderma* spp. with respect to the mode of action against wood-rotting fungi, few studies have been carried out (BRUCE & al., 1984; SILVA & LUPO, 1987: LUPO & SILVA, 1987).

Apart from production of non-volatile metabolites and production of volatile metabolites (DICK & HUTCHINSON, 1966; HUTCHINSON & COWAN, 1972; DENNIS & WEBSTER, 1971b; BRUCE & al., 1984), competition for nutrients (HULME & SHIELDS, 1972) and mycoparasitism (CHET, 1987) were found to be forms of control of certain fungus species by *Trichoderma* spp. The reduction of nutrients has been discussed on the basis of results obtained by BRUCE & KING (1983). The existence of these mechanisms, independently or simultaneously, constitutes a form of reduction of the niche of the involved species.

The present work studies the antagonistic activity of three species of *Trichoderma* and *Gliocladium virens* isolated from natural grassland soil (Canelones, Uruguay). Most of the cultures of wood-rotting fungi were isolated from fruitbodies growing on fallen branches and trunks of native and introduced species (BETTUCCI & GUERRERO, 1971; BETTUCCI, 1987a; PIAGGIO, 1987). *Sistotrema brinkmannii* (Bres.) J. Erikss. was isolated from vegetative mycelium which colonize buried woods of *Abies religiosa* H.B.K. Schl. et Cham. (Parque National, Desierto de Los Leones, Mexico) (BETTUCCI, 1983, 1984) and subsequently identified from their cultures. *Fomes pomaceus* (Pers. ex S.F. Gray) Lloyd was isolated from carpophores developed on the lesions of a standing live peach tree.

MATERIALS AND METHODS

In order to bring out the potential antagonistic effect of non-volatile diffusible metabolites produced by strains of *Trichoderma hamatum* (Bonord.) Bain. (MVHC 5316), *Trichoderma koningii* Oudem. (MVHC 5333), *Trichoderma harzianum* Rifai (MVHC 5318) and *Gliocladium virens* Miller, Gidden and Foster (MVHC 5355), a modified version of the technique proposed by DENNIS & WEBSTER (1971a) was used. Petri dishes containing 18 ml of 2 % agar-malt were utilized. An autoclaved cellophane paper membrane was placed upon the culture medium of fresh antagonistic cultures and 6 mm diameter agar disks were inoculated on the center. These inoculations were carried out with disks obtained from growth area of young cultures of the following wood-rotting Basidiomycetes : *Fomitopsis feei* (Fr.) Kreisel (MVHC 5), *Heteroporus biennis* (Bull. ex Fr.) Sing. (MVHC 106), *Fomes pomaceus* (Pers. ex S. F. Gray) Lloyd (MVHC 5004), *Coriolus pinsitus* (Fr.) Pat. (MVHC 5051), *Gloeoporus dichrous* (Fr.) Bres. (MVHC 5042), *Sistotrema brinkmannii* (Bres.) J. Eriks. (MVHC 96), *Picnoporus sanguineus* (L. Meyer ex Fr.) Murr. (MVHC 5050), *Coriolus versicolor* (L. ex Fr.) Quél. (MVHC 5031), *Laetiporus sulphureus* (Bull. ex Fr.) Murr. (MVHC 5067), *Pheocoriolellus trabeus* (Pers. ex Fr.) Kotl. & Pouz. (MVHC 5138). These inoculations were carried on with disks obtained from the growth area of young cultures.

Growth was measured daily and compared with the control until the latter was found to occupy the maximum diameter of the dish.

Four replicates of the controls and of the previously antagonist-treated dishes were performed. Moreover, in order to assess the effect of the concentration gradient of diffusible metabolite, four disks were respectively placed at 2 cm and 4 cm from the initial inoculum towards the edge of the dish.

Tests were performed at 23°C and pH 4 at the beginning of the experiment.

The multiple comparisons by T method (SCHEFFEE, 1959) was carried out to establish the ordination of antibiotic effect on the growth of tested fungi in relation with the control ones.

The activity of each antagonist with each of the 10 Basidiomycetes species was analyzed separately. Also determined was the effect of the diffusion of metabolites in the culture medium by comparing the growth rate of the disks inoculated at a certain distance from the center in relation with the control and the disk inoculated in the center.

A scale of relative sensitiveness of the Basidiomycetes produced by each antagonist, was developed. The inocula which were completely inhibited by the potential antagonist were transferred to a fresh culture medium to detect any fungicidal activity.

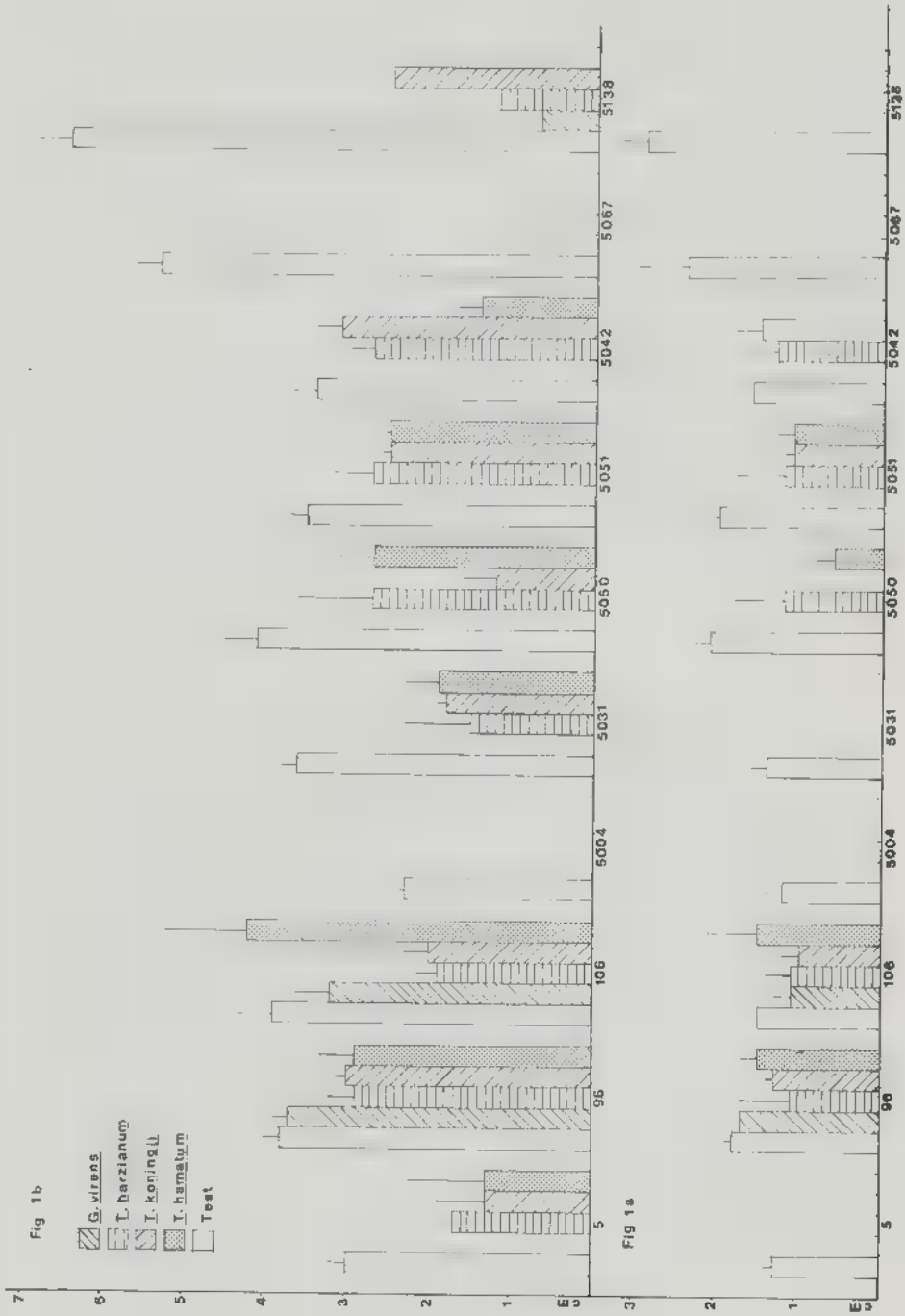
RESULTS

Figure 1a shows that after 48 h of development, of the 10 wood-rotting Basidiomycetes tested, 8 exhibited zero growth with respect to *G. virens*, 7 with *T. hamatus*, 6 with *T. koningii* and 4 with *T. harzianum*. The number of species which failed to develop has decreased to one half or less by the fourth day, except those tested with *G. virens* which present almost the same behaviour (Fig. 1b).

Table 1 shows the percentage of growth inhibition by the diffusible metabolites of *G. virens* and *Trichoderma* spp. when the controls are found to cover the dishes. Evidently the time for covering control Petri dishes is different for each lignivorous species. The most sensitive species to the antagonistic effect were *F. feei*, *F. pomaceus*, *Ph. trabeus* and *L. sulphureus* but they do not exhibit equivalent sensibility to the same antagonists. *L. sulphureus* failed to grow (or showed negligible growth) with the four antagonists. *F. pomaceus* with *G. virens* and *T. hamatum*, *Ph. trabeus* with *G. virens* and *T. koningii* and *F. feei* and *G. dichrous* with *G. virens*.

The T method (SCHEFFEE, 1959) was carried out to assess the differences between the average areas (under the graded curves of growth) of the controls and those submitted to potential antagonists. Growth data were recorded until the control colony covered the maximum diameter of the Petri dish (9 cm).

Heteroporus biennis with *T. hamatum* covered the dish earlier than the control, its growth is stimulated instead of inhibited.



	G. virens	*T. harzianum*	*T. koningii*	*T. hamatum*
F. feei	83.87	48.8	34.4	8.8
S. brinkmannii	0	7.8	5.5	8.8
H. biennis	0	13.3	16.7	*
F. pomaceus	100	68.9	51.1	100
C. versicolor	74.4	32.2	30	13.3
P. sanguineus	61.1	6.6	54.4	*
C. pinsitus	52.2	8.8	10	*
G. dichrous	90	11.1	11.1	26.7
L. sulphureus	100	82.2	100	100
Ph. trabeus	100	58.8	85.5	27.8

Table 1 — Growth inhibition (%) of the Basidiomycetes exposed to metabolites diffusible in the medium (*G. virens* and *Trichoderma* spp.) at the moment when the control attains 9 cm in diameter (it covers the Petri dish). * There is a significant difference in growth by stimulation instead of inhibition.

Tableau 1 — Inhibition de la croissance des Basidiomycètes exposés aux métabolites diffusibles (*G. virens* et *Trichoderma* spp.). Diamètre de la culture témoin : 9 cm. * Différence significative par stimulation et non par inhibition de la croissance.

On the other hand, the growth of *G. dichrous* and *S. brinkmannii* does not significantly differ from the controls when tested with *T. koningii*.

The remaining tested species are significantly inhibited by antagonist with different effectiveness, according to the Basidiomycete and to the antagonist.

In accorcande with the degree of significance of the differences between the growth of controls and the treated ones (Table 2), the most sensitive species were *F. pomaceus*, *L. sulphureus* and *Ph. trabeus*, while the least sensitive were *S. brinkmannii* and *H. biennis*. The remaining species do not differ from one another in their response to the inhibitory activity of the antagonists, considered as a whole.

In turn, *G. virens* was the most efficient inhibitor of growth in most cases analyzed.

No growth was noted when *F. pomaceus* was tested with *G. virens;* when the same disks were transferred to a fresh culture medium without antagonist, they likewise failed to show development of the mycelium.

Figure 1 — Means of the growth diameter (cm) of Basidiomycetes species (confidence interval ± 95 %) within 2 days of inoculation (a) and 4 days of inoculation (b). 5 : *F. feei*, 96 : *S. brinkmannii*, 106 : *H. biennis*, 5004 : *F. pomaceus*, 5031 : *C. versicolor*, 5050 : *P. sanguineus*, 5051 : *C. pinsitus*, 5042 : *G. dichrous*, 5067 : *L. sulphureus*, 5138 : *Ph. trabeus*.

Figure 1 — Croissance moyenne (diam. en cm) des Basidiomycètes (intervalle de confiance ± 95 %) 2 jours (a) et 4 jours (b) après l'inoculation.

	G. virens	*T. harzianum*	*T. koningii*	*T. hamatum*
F. feei	4	1	1	1
S. brinkmannii	0	1	0	1
H. biennis	2	1	1	*
F. pomaceus	4	3	3	4
C. versicolor	3	2	1	1
P. sanguineus	3	1	2	1
C. pinsitus	3	1	2	1
G. dichrous	4	1	0	2
L. sulphureus	4	4	4	4
Ph. trabeus	4	3	4	3

Table 2 – Growth inhibition ranked in the values of the significant differences. 0 : no effect of antagonist (no significant difference), 4 : maximum effect of the antagonist (zero or negligible Basidiomycete growth).

Tableau 2 – Inhibition de la croissance ordonnée selon les valeurs des différences significatives. 0 : effet nul de l'antagoniste (pas de différence significative), 4 : effet maximal de l'antagoniste (croissance nulle ou négligeable des Basidiomycètes).

	G. virens			*T. harzianum*			*T. koningii*			*T. hamatum*		
	0	2	4	0	2	4	0	2	4	0	2	4
F. feei	100	100	100	100	100	0	100	100	0	100	33	0
S. brinkmannii	2.86	14.3	14.3	64.6	25.7	14.3	25.7	37.1	16	13.1	21.7	5.7
H. biennis	28.7	0	[illegible]	28.7	5.33	0	35.3	22	[illegible]	[illegible]	[illegible]	0
F. pomaceus	100	100	0	100	100	10.3	100	100	8.5	100	100	0
C. versicolor	100	100	0	100	100	1.4	100	8.4	0	100	19	0
P. sanguineus	100	100	41	43.4	3.3	0.9	100	100	32.7	100	21.2	9.4
C. pinsitus	100	100	[illegible]	42.1	7.4	0	48	33.2	1	44.6	25.7	0
G. dichrous	100	100	[illegible]	22.8	3.1	0	7.4	10.5	[illegible]	100	34	0
L. sulphureus	100	100	42.6	100	100	54.9	100	100	42.6	100	100	[illegible]
Ph. trabeus	100	100	22.1	100	100	100	100	100	51.9	100	46	31.1

Table 3 – Growth inhibition of Basidiomycetes (%), when the inoculum disks are placed in the center (0), 2 and 4 cm from the center. Growth of the control was considered as 100 %.

Tableau 3 – Inhibition de la croissance des Basidiomycètes (%) lorsque l'inoculum est placé au centre (0), à 2 et 4 cm du centre. Croissance du témoin : 100 %.

On the other hand, the inoculum disk of *P. sanguineus* tested with *G. virens* became stained more intensely than do the control, during the first 48 hours. After this delay the mycelium started to grow. Apparently this growth was allowed by the decrease of metabolites concentration in the central zone around the disk.

Generally at hour 48, disks inoculated far from the dish center, appeared less affected by the inhibitory activity than those of the center, the more so with

greater distance. By contrast, *S. brinkmannii* tested with *G. virens*, as well as with *T. hamatus* and *T. koningii*, was inhibited somewhat closer to the edge of the dish than to its center. The inocula lying in the center were less inhibited than those placed within 2 cm. This fact remains unexplained. Moreover, *P. sanguineus* and *L. sulphureus* exhibited a marked inhibition at the innermost half of disk inoculated within 4 cm while the outermost half was less affected when tested with *G. virens* and *T. koningii*. *F. pomaceus* mycelium placed within 2 cm grew outwardly and upwardly, starting from the fourth day of inoculation with *T. harzianum* and the fifth day with *T. koningii*. The outermost disks of *F. pomaceus*, with *G. virens*, start growth eleven days after inoculation; disks placed within 2 cm had been totally inhibited in their growth.

Inhibition was calculated by means of $I = \frac{B \times 100}{T} - 100$, B being the mean of the diameters of the Basidiomycete colonies tested with a potential antagonist and T the mean of the diameters of the control colonies (Table 3).

DISCUSSION

The discussion hinged fundamentally upon the results obtained within the 48 hours following the inoculation of the tested species, as it was deemed that the antagonistic effect is affected by the diffusion of metabolites and by the discontinuance of their production following the elimination of the antagonistic species from the culture medium.

The species of wood-rotting fungi tested exhibit variable sensitivity to the inhibitory effect of diffusible metabolites of *Trichoderma* spp. and *G. virens*. It should be stressed that, at the same concentration, the same antagonist affects the different Basidiomycetes in a different manner.

The efffect of concentration may be different, fungistatic or fungicidic. While at high concentration *G. virens* was fungicidic for *F. pomaceus*, with *L. sulphureus* it behaved as fungistatic. This latter fungus, following decrease of concentration, begins to grow while *F. pomaceus* does not, even when transferred to a medium without an antagonist. This is in agreement with other reports (DENNIS & WEBSTER, 1971a; BRUCE & al., 1984) in which it is stated that the antagonistic effect is species-specific.

Moreover, as pointed out by DENNIS & WEBSTER (1971a) not all the strains of the same antagonistic species are equally efficient.

The growth rate is not only manner in which the Basidiomycetes are able to overcome the antagonistic effect but it is a liable mean evaluation of antagonistic activity. This effect becomes manifest with similar intensity in *F. pomaceus* and *L. sulphureus*. Both species present a different growth rate; the former, slow, the latter, very fast. On the other hand, *S. brinkmannii*, which exhibits a comparatively fast growth does not practically present sensitivity to antagonists.

The effect of diffusion of metabolites, as seen in Table 3, is a marked one, since in 65 % of cases sensitiveness was maximal in the center and within 2 cm,

48 hours after inoculation, and declined or dropped to zero within 4 cm.

L. sulphureus is the most sensitive species at the lowest concentration as it is inhibited by three of the four antagonists (*G. virens, T. harzianum, T. koningii*) between 42 % and 55 % of its growth 4 cm from the center. On the other hand *G. virens* has a fungicidic effect upon *F. pomaceus* but only at the center or 2 cm from the inoculum of the antagonist. It was furthermore seen that *H. biennis* was stimulated in the presence of *T. hamatum.*

HULME & SHIELD (1970) postulated that in the case of *G. virens* the effect of the diffusible substances does not have an important ecological significance; what confer a greater competitive ability to this fungus is its high growth rate. In the condition tested we cannot suggest competition for nutrients because the antagonist does not coexist with the Basidiomycete; there only remain the diffusible substances and, morevover, this assertion is conforted by the fact that *F. pomaceus* and *L. sulphureus* have the same behaviour independently of their growth rates.

These results and those obtained by BRUCE & al. (1984) therefore contradict interpretations by HULME & SHIELD (1972) as the only form of competition. The decrease of nutrients in the medium, owing to competition between organisms with different growth rates may be an indirect cause of inhibition, as a result of involvement of the metabolism of the handicapped species.

Preliminary observations of coexistence of *S. brinkmannii* with *Trichoderma* spp. (BETTUCCI, 1983, 1985, 1987b) are suggestive of the lack of sensitivity of Basidiomycete with respect to diffusible metabolites in the medium. The high colonizing ability of buried woods, under laboratory conditions, may have been favoured by a «cascade» process analogous to that described by SMITH & al. (1981). Also to be added is the ability of *G. virens* and of several species of *Trichoderma* to produce volatile metabolites with an inhibiting effect upon the growth of other species (DENNIS & WEBSTER, 1971b).

It has been possible to observe a marked difference between the antagonistic activity of *G. virens* with species of *Trichoderma,* contrarily to remarks of DENNIS & WEBSTER (1971a).

ACKNOWLEDGEMENT

The authors would like to thank Dr. Gonzalo Pérez for the statistical analysis of data and his helpful criticism of this paper.

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